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DNA Sequences Responsible for Specificity of DNA Packaging and Phage Growth

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T3 and T7 phages package recombinant plasmids carrying DNA necessary for DNA packaging (the *pac* sequences) of T3 and T7, respectively. Packaging is specific between T3 and T7. The *pac* sequence has a bipartite structure, consisting of target sequences for processing of concatemeric DNA (*pac* C) and its left side flanking sequence containing a promoter for phage RNA polymerase (*pac* B). To determine the sequences responsible for the specificity of plasmid DNA packaging, plasmids chimeric for the *pac* B and *pac* C sequences of T3 and T7 were constructed. Analysis of packaging of the chimeric plasmid DNAs showed that *pac* B is responsible for the packaging specificity of T3 and T7 DNAs. Plasmids carrying the genetic right end of T3 and T7 DNA interfered with the growth of T3 and T7 phages, respectively. Interference was specific between T3 and T7. *pac* B and sequences between *pac* B and *pac* C, but not *pac* C, were responsible for the interference. The specificity of interference was determined by *pac* B and sequences responsible for interference were partially defined.

INTRODUCTION

The termini of mature DNA from T3 and T7 phages contain the same 230 and 160 base pair (bp) sequences, respectively, called terminally redundant sequences (T7, Dunn and Studier, 1983: T3, Fujisawa and Sugimoto, 1983). Phage DNA is synthesized as concatemers in which unit length molecules are joined together in a head-to-tail fashion through the terminally redundant sequences. During packaging of DNA, mature monomers are cut from the concatemer. Although T3 and T7 phages are closely related, a crude *in vitro* DNA packaging system, composed of extracts prepared from cells infected with T3 or T7 phage, encapsidates homologous DNA more efficiently than heterologous DNA (Fujisawa and Yamagishi, 1981). As shown in a previous paper (Hashimoto and Fujisawa, 1988), T3 phage packaged recombinant plasmid DNAs carrying DNA sequences necessary for packaging of T3 DNA (the *pac* sequence) but T7 phage could not package these plasmid DNAs, indicating that T3 and T7 *pac* sequences can be discriminated during packaging of recombinant plasmid DNAs. The *pac* sequence has a bipartite structure, consisting of target sequences for processing of concatemeric DNA (*pac* C) and its left side flanking sequence for recognition by the packaging machinery (*pac* B). The *pac* B sequence contains a promoter for T3 RNA polymerase (*pac* B promoter) and a following GA stretch located at the genomic right end (Hashimoto and Fujisawa, 1992a). The same bipartite structure is proposed as the *pac* sequence for T7 (Chung and Hinkle, 1990). Deletion experiments with *pac* B indicate that promoter activity is necessary for plasmid DNA packaging (Hashimoto and Fujisawa, 1992a). T3 and T7 RNA polymerases strictly recognize their own promoters (Dunn *et al.,* 1971). From these results, we concluded that transcriptional specificity is responsible for the packaging specificity. The conclusion is supported by the observation that the crude *in vitro* DNA packaging systems packaged heterologous DNA as well as homologous DNA, provided the heterologous phage RNA polymerases were added (Hashimoto and Fujisawa, 1992b).

In this paper, we describe experiments to determine the sequences responsible for the specificity of plasmid DNA packaging. We constructed chimeric plasmids of *pac* B and *pac* C between T3 and T7. The specificity analysis of packaging of the chimeric plasmid DNAs indicates that *pac* B is responsible for the packaging specificity of T3 and T7 DNAs. As observed with T7 by Chung and Hinkle (1990), we find that plasmids carrying the genetic right end of T3 DNA interfere with the growth of T3 phage. The interference is specific between T3 and T7. We define partially the sequences responsible for the interference and for its specificity.

MATERIALS AND METHODS

Bacteria, phages, and plasmids

Escherichia coli R11S (su^+), ER22 (su^-), and JM107 (F, su^+) were used for transduction experiments by T3 and

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T7 phages. T3 wild type and 3^- (endonuclease)·17.5^- (lysis) are from our laboratory stocks. T7 wild type and 3^- (endonuclease) were generously provided by Dr. F. W. Studier.

Medium

M9A medium is M9 medium supplemented with 0.1% casamino acids.

Construction of plasmids with defined restriction fragments spanning *pac* sequences of T7 and T3 DNAs

Using appropriate restriction sites, p7B7C642 was constructed by inserting a 915-bp fragment (799−−116) into pUC18 (Fig. 1A). The deletion series of p7B7C, p7B, and p7C was constructed from p7B7C642 by using appropriate restriction enzyme sites as shown in Fig. 1A. pBKS7C189 or pBSK7C189 was constructed by inserting a 305-bp *Dra*I–*Eco*RI fragment into the *Hin*dIII–*Eco*RI sites of pBluescript KS or SK, respectively. p ϕ137C642 was constructed by inserting a 465-bp *Cla*I–*Nru*I fragment carrying the ϕ13 promoter of phage T7 (Dunn and Studier, 1983) into p7B7C642 in place of *pac* B in the same transcriptional orientation (Fig. 2, line 2). Plasmids carrying T3 *pac* sequences were constructed from p3B3C683 by using appropriate restriction sites (Fig. 1B) (see Hashimoto and Fujisawa, 1992a). T3/T7 chimeric plasmids were constructed from p3B3C683 and p7B7C642 by using appropriate restriction sites (Fig. 1C).

Assays for plasmid transduction

Plasmid transduction was performed according to Hashimoto and Fujisawa (1988). Briefly, R11S cells carrying appropriate plasmids at a density of 5×10^8 cells/ml in M9A medium were infected with T3 3^-·17.5^- or T7 3^- phage at a multiplicity of infection of 7 at 30°. Cells were lysed 60 min after infection. For assay of transduction by T3 or T7 phage, a 0.2-ml aliquot of a recipient strain, ER22 or JM107, respectively, was added with a sample containing transducers (50 μl) and, after incubation for 30 min at 30°, cells were spread on an LB agar plate containing ampicillin (25 μg/ml) and incubated for 24 hr at 37°. ER22 or JM107 was used to restrict the growth of T3 or T7 phage in the sample, respectively, because T3 and T7 phages could propagate and infect transformants during transformation (the latent period of T3 and T7 phages was about 25 min at 30°).

TABLE 1

Transduction of Recombinant Plasmids Carrying T3 *pac* Sequences by T3 and T7 Phages

	T3	T7
p3B3C683	2.8×10^7	7.2×10^4
p3B3C429	5.5×10^7	4.0×10^4
p3BC140	6.5×10^7	3.5×10^4
pUC18	4.7×10^4	2.2×10^4

Note. Constructs of recombinant plasmids are presented in Fig. 1B. The transducing activity was assayed as described under Materials and Methods. The results represent an average of transducers per milliliter obtained in two to three experiments.

TABLE 2

Transduction of Recombinant Plasmids Carrying T7 *pac* Sequences by T3 and T7 Phages

	T7	T3
p7B7C642	2.7×10^8	2.9×10^6
p7B7C189	5.4×10^8	1.9×10^6
p7B7C119	2.3×10^8	3.8×10^5
p7B119	3.9×10^5	ND
p7B309	2.5×10^5	ND
p7B477	4.9×10^5	ND
p7B576	3.3×10^5	ND
p7B	7.2×10^5	ND
p7C642	4.0×10^4	ND
p7C189	1.5×10^4	ND
pUC18	1.5×10^4	4.7×10^4

Note. Constructs of recombinant plasmids are presented in Fig. 1A. The transducing activity was assayed as described under Materials and Methods. The results represent an average of transducers per milliliter obtained in two to three separate experiments. ND, not determined.

Assays for phage yields

R11S cells carrying *pac* sequences of T3 or T7 phages at a density of 5×10^8 cells/ml in M9A medium were infected with T3 or T7 wild-type phage at a multiplicity of infection of 7 at 37°. Thirty minutes after infection, a few drops of chloroform were added to the culture to complete lysis and phage yields were assayed by titration on R11S.

RESULTS

T3/T7 sequences responsible for specificity of DNA packaging

The *pac* sequence of T3 phage is composed of the *pac* B sequence, including a promoter sequence for phage RNA polymerase located at the right end of T3 genomic DNA, and the *pac* C sequence, the target sequence for concatemer processing. The same bipartite structure was proposed for the *pac* sequences of T7 (Chung and Hinkle, 1990). As shown in Table 1, we confirmed the previous results that plasmids carrying *pac* B and *pac* C sequences were efficiently packaged by T3, but not by T7 phage (Hashimoto and Fujisawa, 1988) and that the intervening sequences between *pac* B and *pac* C (the I sequence) were not necessary for efficient packaging of plasmid DNA (Hashimoto and Fujisawa, 1992a).

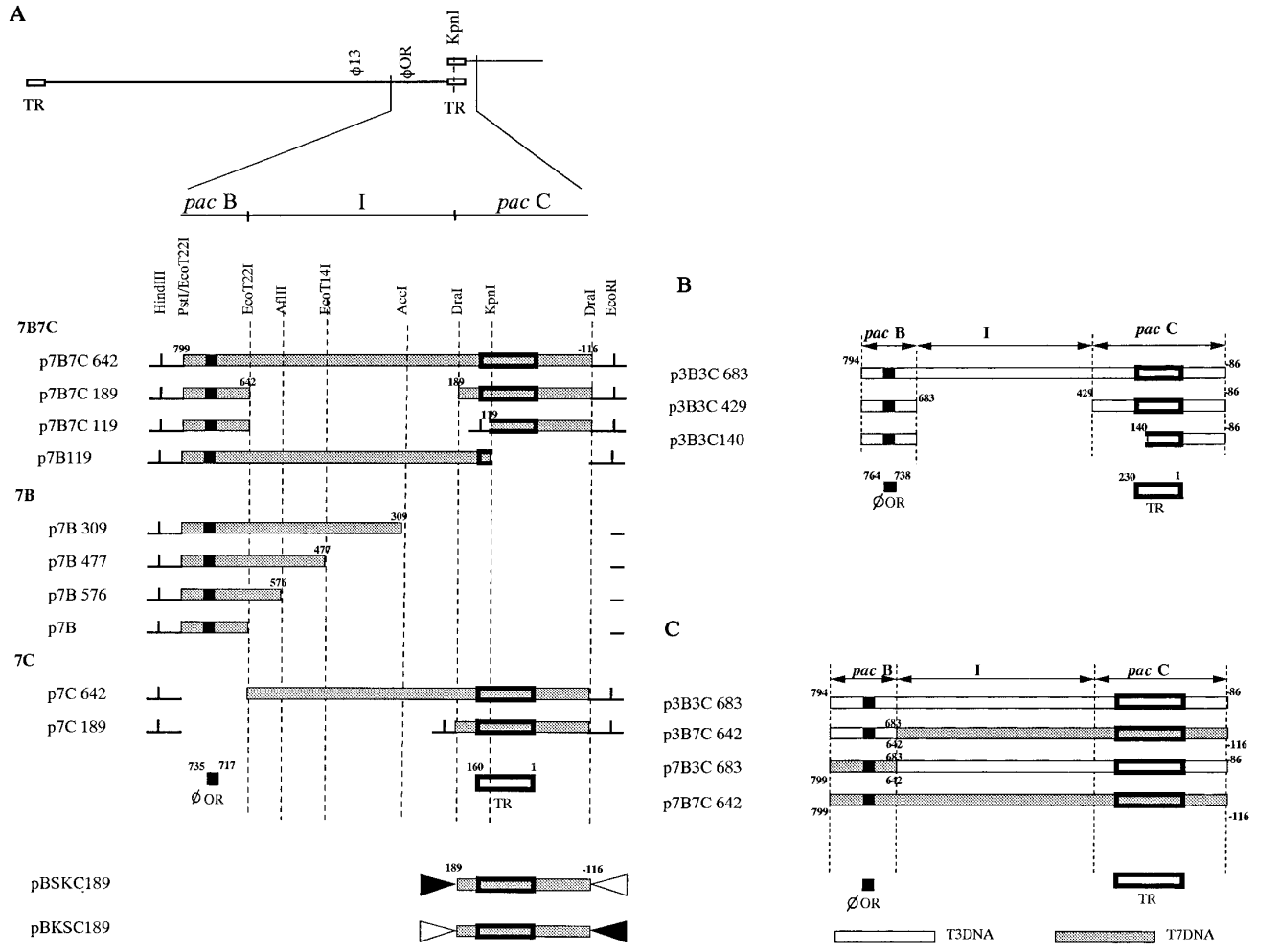

FIG. 1. A schematic presentation of T3 and T7 DNA fragments cloned into pUC18. Open and shaded boxes represent T3 and T7 sequences, respectively. The numbers above and under boxes are the nucleotides numbered leftward or rightward (−) from the genetic right end of T3 or T7 phages, respectively. Heavy-lined boxes indicate the terminally redundant sequences (TR). *pac* B promoter is represented as ϕOR. T3 or T7 promoter of pBluescript vector is presented by open or shaded arrowhead, respectively, and directions of the arrowheads indicate the orientation of transcription from the promoters. Restriction enzyme sites are indicated. (A) T7 constructs, (B) T3 constructs, (C) T3/T7 chimeric constructs.

With T3 phage, the left border of the concatemer joint was not required for efficient packaging as long as the right 140-bp terminally redundant sequence remained (Table 1, p3B3C140) as shown in a previous paper (Hashimoto and Fujisawa, 1992a).

With T7, plasmids carrying the *pac* B and *pac* C sequences were efficiently packaged by T7, but not by heterologous T3 phage (Table 2, p7B7C642) and the I sequence was not necessary for efficient packaging of plasmid DNA (p7B7C189) as observed with T3 (Table 2). A plasmid carrying a short deletion of the left border sequence did not show reduced packaging efficiency (Table 2, p7B7C119). Deletions removing *pac* B completely lost packaging activity (Table 2, p7C series).

As shown above, plasmids carrying the *pac* B and *pac* C sequences of T3 or T7 were recognized by homologous phages but not well by heterologous phages, indicating that these plasmids carry sequences responsible for packaging specificity between T3 and T7. To determine the sequences responsible for the packaging specificity, we constructed plasmids carrying chimeric *pac* sequences between T3 and T7 (Fig. 1C) and examined their packaging efficiency. As shown in Table 3, a chimeric plasmid carrying T3 *pac* B and T7 *pac* C (p3B7C642) was packaged by T3 phage as efficiently as a plasmid containing T3 *pac* B and *pac* C (p3B3C683) but was not packaged by T7 phage. On the other hand, a chimeric construct containing T7 *pac* B and T3 *pac* C (p7B3C683) was packaged by T7 phage as efficiently as a plasmid containing T7 *pac* B and *pac* C (p7B7C642) but was not packaged by T3. These results demonstrate that *pac* B is responsible for DNA packaging specificity between T3 and T7. The derivatives of *pac* C did not affect the transduction efficiency at all by either phage, although *pac* C was essential for efficient transduction of recombinant plasmids in concert with *pac* B. When a T7 *pac* C sequence was inserted into pBluescript SK and KS,

TABLE 3

Transduction of Recombinant Plasmids Carrying T3/T7 Chimeric *pac* Sequences by T3 and T7 Phages

	T3	T7
p3B3C683	2.8×10^7	7.2×10^4
p3B7C642	6.9×10^7	6.3×10^4
p7B3C683	1.3×10^6	3.8×10^8
p7B7C642	2.9×10^6	1.8×10^8
pUC18	4.7×10^4	2.2×10^4
pBSKC189	9.2×10^4	3.2×10^7
pBKSC189	6.4×10^6	2.2×10^4
pBluescript SK	1.7×10^4	2.1×10^4

Note. Constructs of recombinant plasmids are presented in Fig. 1C. The transducing activity was assayed as described under Materials and Methods. The results represent an average of transducers per milliliter obtained in two to three experiments.

in which T3 and T7 promoters are oriented in opposite directions (Fig. 1A), the T3 or T7 promoter was utilized for efficient packaging only in the same orientation relative to the concatemer junction as the *pac* B promoter (Table 3).

T3/T7 sequences responsible for interference of T3 and T7 phage growth

Plasmids carrying sequences from the right end of the T7 genome interfere with the growth of T7 (Campbell *et al.,* 1978; Chung and Hinkle, 1990). It is interesting to examine whether T3 growth is similarly interfered with by plasmids carrying the corresponding T3 sequences and whether the interference is specific between T3 and T7. Cells carrying recombinant plasmids were infected with T3 or T7 phage and phage yields were measured. As shown in Table 4, plasmids carrying sequences from the right end of the T3 or T7 genome interfered with the growth of homologous phage, although the interference with T3 phage was less severe than that with T7 phage. Both the *pac* B and I sequences were essential for the interference but intact *pac* C was not required (Fig. 2, lines 9, 16, and 17), although Chung and Hinkle (1990) reported that *pac* B by itself interfered with growth of T7. To define the region responsible for the interference in the I sequence, deletions of the I sequence were constructed and examined for interference with T7 phage growth, which was more severe than that of T3. The I sequence was tentatively divided into five subregions, I to V (Fig. 2, line 1). Subregions I+II+III interfered with the growth of T7 as well as the intact I sequence (Fig. 2, lines 1 and 7), but region I was inactive in the interference (Fig. 2, lines 9 and 10), indicating that subregions II and III are primarily involved in the growth interference. The interference by I+III+IV+V (line 12), III+IV+V (line 13), and IV+V (line 14) was almost the same as that by V (line 15), suggesting that subregions I, III, and IV were not involved in the interference. However, subregion III was necessary for severe interference in conjunction with subregion II because the interference of subregions I+II+III decreased when subregion III was deleted (lines 7 and 8). When subregions I+II+III were reversed, the interference decreased to the same extent as that by subregion IV+V (lines 5, 6, and 14), suggesting that the orientation of subregion II+III is important for the interference.

As shown in Table 4, the interference was specific between T3 and T7. To define sequences responsible for the specificity of the growth interference, the phage yields were determined with chimeric plasmids carrying *pac* B and I sequences between T3 and T7. As shown in Table 5 and Fig. 2, line 4, phage growth was interfered with by plasmids carrying homologous *pac* B, independently of the derivation of the I sequence, indicating that the *pac* B sequence is primarily responsible for the specificity of the growth interference. Although the T7 ϕ13 promoter and the Bluescript T3 and T7 promoters were active in DNA packaging, the former interfered moderately with phage growth and the Bluescript promoters were inactive in the interference.

DISCUSSION

The present results confirm the previous conclusions that the *pac* sequence of T3 phage is composed of *pac* B, including a promoter in the genetic right end and *pac* C, the target sequence for concatemer processing, and that the I sequence between *pac* B and *pac* C is not required for packaging (Hashimoto and Fujisawa, 1992a). The sequence necessary for generation of the genetic left end in the *pac* C sequence was not required for efficient packaging by T7 as observed for T3 (Hashimoto and Fujisawa, 1992a; this paper), consistent with the observations by Chung and Hinkle (1990). In the right end of T3 and T7 DNAs, short inverted repeat sequences are found at a position centered around 417 and 350 nucleotides from the right end, respectively (Fig. 3), named the M-palindrome by Chung *et al.* (1990). The M-

TABLE 4

Effect of T3 and T7 *pac* Sequences on the Growth of T3 and T7 Phages

	T3	T7
p3B3C683	9.8×10^8	1.7×10^{10}
p3B3C140	2.3×10^{10}	ND
p3B429	1.6×10^9	ND
p7B7C642	3.3×10^8	4.1×10^{10}
p7B7C119	1.1×10^{10}	4.6×10^{10}
pUC18	3.6×10^{10}	3.5×10^{10}

Note. R11S carrying *pac* sequences of T3 or T7 phages were infected with T3 or T7 and phage yields were assayed as described under Materials and Methods. The results represent an average of PFU/ml obtained in two or three separate experiments. ND, not determined.

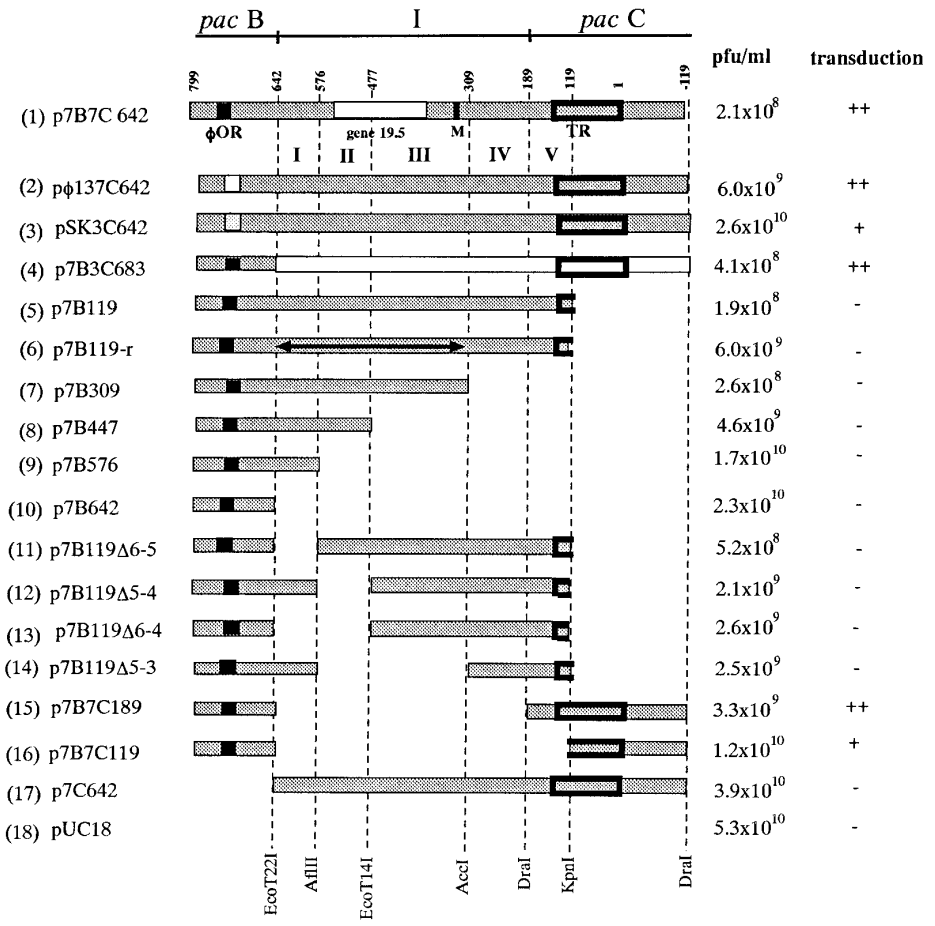

FIG. 2. A schematic presentation of T7 DNA fragments cloned into recombinant plasmids and the growth interference of T7 phage by recombinant plasmids. (1) The numbers are the nucleotides numbered leftward or rightward (−) from the genetic right end of T7 phage DNA. The positions of *pac* B promoter (ϕOR), gene 19.5, M-palindrome (M), and the terminally redundant (TR) sequences, and restriction enzyme sites are indicated. (2) The open box indicates T7 ϕ13 promoter in place of T7 *pac* B promoter. (3) The shaded box indicates pBluescript T7 promoter in place of T7 *pac* B promoter. (4) The open box indicates the T3 counterpart in place of the corresponding region of T7. (5–17) Deletion series of T7 sequences. Recombinant plasmids were assayed in R11S for production of T7 phage as described in Table 4. The results represent an average of PFU/ml obtained in two to three experiments. Transduction efficiency is shown in Tables 2 and 3.

palindrome sequences are conserved between T3 and T7. This inverted repeat formed a hairpin structure in the T7 concatemer (Chung *et al.,* 1990). Chung *et al.* (1990) proposed that the hairpin structure may play a role in duplicating the terminal repeat for packaging, specifically in the formation of the left end, and may also serve to signal the packaging apparatus where right and left ends are to be located on the T7 concatemer. However, the M-palindrome was not required for efficient plasmid packaging by either T3 or T7 phage (Tables 1 and 2). Independence of the M-palindrome is not special for plasmid DNA packaging, because T3 and T7 mutants, deleting the M-palindrome, are viable and their burst sizes are the same as those of wild-type phages (our unpublished results).

TABLE 5

Effect of T3/T7 Chimeric *pac* Sequences on the Growth of T3 and T7 Phages

	T3	T7
p3B3C683	9.8×10^8	1.7×10^{10}
p3B7C642	1.9×10^9	1.2×10^{10}
p7B3C683	3.4×10^{10}	4.1×10^8
p7B7C642	3.8×10^{10}	3.3×10^8
pUC18	3.5×10^{10}	3.6×10^{10}

Note. R11S carrying *pac* sequences of T3 or T7 phages were infected with T3 or T7 and phage yields were assayed as described under Materials and Methods and represent an average of PFU per milliliter obtained in two or three separate experiments.

T3 and T7 phages package homologous DNA more efficiently than heterologous DNA. In previous papers (Hashimoto and Fujisawa, 1992a, b), we showed that promoter activity is necessary for plasmid DNA packaging and that phage RNA polymerases play a crucial role in determination of the DNA packaging specificity. Analysis of the packaging specificity of chimeric plasmids of *pac* B and *pac* C between T3 or T7 clearly demonstrate

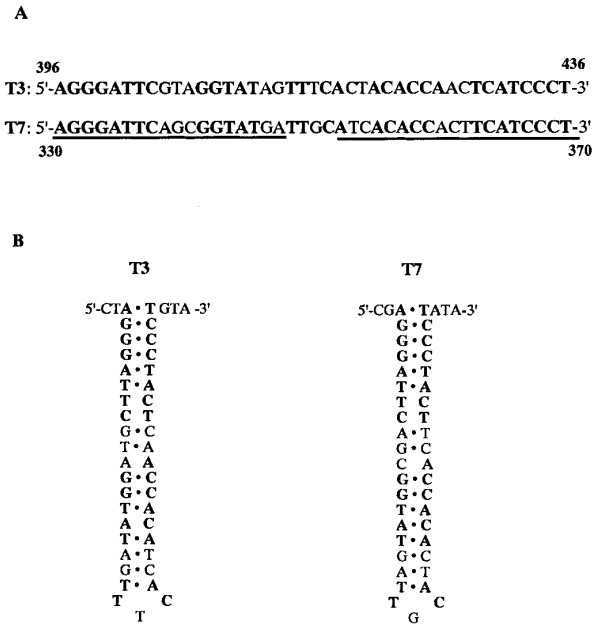

FIG. 3. Nucleotide sequences of M-palindromes of T3 and T7 phages. (A) Nucleotide sequences of the M-palindromes of T3 and T7 phage located in the genetic right end are shown. Numbers are nucleotides numbered leftward from the genetic right end. Sequences constructing the stem part are underlined. Conserved nucleotides are indicated by boldface. (B) Diagrams showing possible structures for the hairpin M-end of T3 (left) and T7 phages (right). T3 and T7 sequences are from Yamada *et al.* (1986) and Dunn and Studier (1983), respectively.

that *pac* B is responsible for DNA packaging specificity. When the T7 *pac* C sequence was inserted between the T3 and T7 promoters of Bluescript plasmids, the promoters, oriented to transcribe rightward through the concatemer junction, were used for efficient plasmid packaging by homologous phages. These observations support the previous conclusion that the transcriptional specificity determines the packaging specificity of T3 and T7 (Hashimoto and Fujisawa, 1992b).

According to Chung and Hinkle (1990), the *pac* B sequence by itself was sufficient for growth interference of T7. However, we found that *pac* B alone was not sufficient for growth interference of T3 and T7. These discrepancies remain to be explained. Deletion analyses showed that the sequence responsible for the interference was composed of two domains, *pac* B and I sequences. Deletion studies with the I sequence of T7 indicated that the sequences responsible were primarily located in subregions II+III, while subregion V moderately interfered with phage growth. The former region includes gene 19.5, which is homologous between T3 and T7 (61.2% in amino acid sequences and 74.8 in nucleotide sequences) (Yamada *et al.,* 1986). Gene 19.5 is not essential for phage growth of T3 phage because a mutant deleting gene 19.5 is viable (our unpublished results). Inversion of this sequence decreased interference and the remaining interference appears to be due to subregion V, suggesting that gp19.5 might be involved in the interference. Since significant interference remained after deletion of the C-terminal half of gp19.5, it is possible that not gp19.5 but the sequence of the region is responsible for the severe interference.

Packaging and growth interference by recombinant plasmids are common in their requirement for the *pac* B promoter and the involvement of *pac* B in their specificities. However, *pac* B appears to function in different manners in these processes because, although the T7 promoter in pBluescript and the ϕ13 promoter were active in plasmid DNA packaging (Table 3), the former did not work at all in the interference assay and the latter was only moderate in the interference assay. Analysis of the inhibitory effect on phage growth is in progress.

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